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Pharmacokinetic Model of Target-Mediated Disposition of Thrombopoietin

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ABSTRACT

Thrombopoietin, TPO, a 353 amino acid cytokine, is a primary regulator of platelet production that was cloned recently. A target-mediated (platelet receptors) pharmacokinetic model was developed to characterize the disposition of TPO. Receptor-mediated endocytosis was assigned as the major elimination pathway in the model. A nonspecific binding compartment was also incorporated into the model. TPO concentration vs time profiles from a published phase 1 and 2 clinical trial were used to apply this model. Noncompartmental analysis demonstrated that TPO exhibits nonlinear kinetics. The proposed model captured the concentration-time profiles relatively well. The first-order internalization rate constant was estimated as 0.1 h^{-1}. The endogenous binding capacity was estimated as 164.0 pM. The second-order binding association constant (k_{on}) was 0.055 $h^{-1} \cdot pM^{-1}$ and the first-order dissociation constant (k_{off}) was estimated as 2.5 h^{-1}, rendering the equilibrium dissociation constant K_d as 45.5 pM. This model may be relevant to other therapeutic agents with receptor-mediated endocytotic disposition.

KEYWORDS: thrombopoietin, receptor-mediated drug disposition, pharmacodynamic model

INTRODUCTION

During the process of platelet production, hematopoietic growth factors play a key regulatory role. Among them, thrombopoietin (TPO) is the primary regulator in megakaryocytic lineage. Although the existence of TPO was suggested early in the 1960s, it was not identified until 1994 when 5 different groups successfully cloned the cDNA of TPO independently.[1-5]

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The essential event that initiated the cloning of TPO was the identification of its receptor: c-Mpl.[6] Human c-Mpl receptor is a transmembrane receptor that belongs to the cytokine receptor superfamily as characterized by its double hemopoietin receptor domain with 2 pairs of conserved cysteine residues. The expression of c-Mpl appears to be restricted to platelets, megakaryoctes, and CD34+ progenitor cells.[7]

Upon binding to its receptor and undergoing subsequent cellular signal transduction processes such as tyrosine phosphorylation of TPO receptor, TPO stimulates the proliferation, differentiation, and maturation of megakaryocytes. In vitro studies have demonstrated that TPO increases the ploidy and number of megakaryocytes as well as stimulates the expression of platelet-specific markers such as Ib and IIb/IIIa and promotes endomitosis in megakaryocytes. TPO is also a potent megakaryocyte colony-stimulating factor in the early stage of megakaryocytopoiesis, capable of inducing the proliferation of megakaryocyte progenitor cells.[8] In addition, TPO acts in synergy with erythropoietin to promote the growth of erythroid progenitor cells.[9] In the presence of early-acting cytokines such as IL-3 or steel factors, it exhibits both proliferative and differentiative effects on primitive hematopoietic stem cells.[10]

The previous results from in vitro studies have been confirmed in preclinical studies. Administration of TPO to normal mice caused a 4-fold rise in platelet count, along with a 20-fold increase in marrow colony-forming unit-megakaryocytes and a 10-fold increase in megakaryocytes. Panhematopoietic effects were also found.[11] In myelosuppressed animal models, TPO ameliorates thrombocytopenia via increasing the platelet count nadir and accelerating platelet recovery.[12]

TPO is produced predominantly in the liver by hepatocytes. Other organs expressing TPO mRNA are bone marrow, kidney, brain, testis, and spleen.[13-14] The circulating level of free TPO is inversely related to the platelet count.[15] By analogy to the transcriptional regulation of erythropoietin in anemia,[16] it was suggested that circulating TPO concentrations might be regulated by the TPO gene expression level. However, in animals with severe

thrombocytopenia, endogenous TPO mRNA level is normal.[17] As an alternative, the TPO level might be regulated by binding to its receptors on platelets. In thrombocytopenic animals, plasma TPO concentrations drop soon after platelet transfusion and rise after platelet count drops.[15]

The concept of target-mediated drug disposition has been introduced previously[18] and explored further recently.[19] In this study, we applied a target-mediated drug disposition pharmacokinetic model for TPO and investigated the nonlinear kinetic properties of TPO kinetics.

MATERIALS AND METHODS

Theoretical

Based on its physiology, a mechanistic pharmacokinetic model for TPO is proposed and shown in Figure 1. Circulating free TPO (TPO_f) and c-Mpl receptors (R_p) on the platelet surface are constantly produced at zero-order rates denoted by k_t and k_r. The whole model is composed of 2 major binding components to account for TPO disposition. One component is that TPO binds to c-Mpl receptor on platelet surface. This mechanism is described by the second-order association constant k_{on} and the first-order dissociation constant k_{off} ($K_d = k_{off}/k_{on}$). This binding process is capacity limited, with the equilibrium constant K_d ranging between 100 and 846 pM.[20-22] After free TPO in the circulation binds to c-Mpl, a drug-receptor complex (TPO_p) is formed, which then dissociates back to free drug and receptor. This complex can also be internalized into the platelet and degraded by lysosomes.[21] The internalization is described by the first-order constant k_{int}. This process, also known as receptor-mediated endocytosis, reflects the major elimination pathway of TPO. After internalization and degradation, the c-Mpl receptor will not recycle back to the platelet surface.[20-21]

Since the binding of TPO to the c-Mpl receptor would stimulate the production of platelets, the production rate of free receptor would be concomitantly increased. The model assumes that this production rate is proportional to the concentration of drug-receptor complex (TPO_p), which is reflected by the equation below:

$$\text{Free receptor production rate} = k_r \cdot TPO_p / TPO_{p,0} \quad (1)$$

where $TPO_{p,0}$ represents the baseline level of TPO bound to c-Mpl. Equation 1 is driven by minimizing of the number of parameters that would be necessary if a more physiological saturable stimulation of the c-Mpl production was introduced.

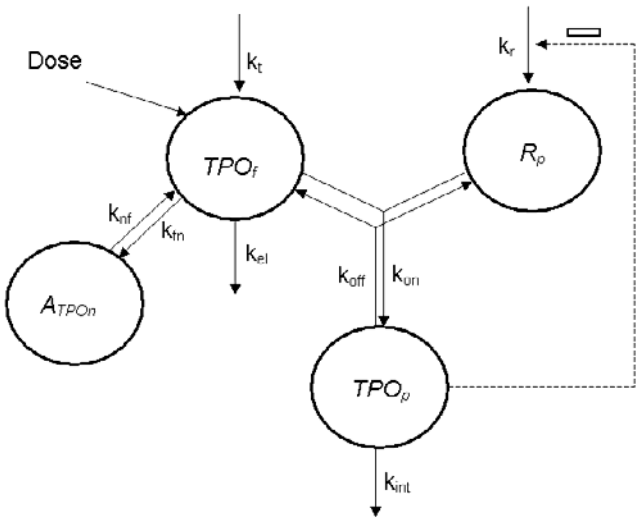

Figure 1. Receptor-mediated disposition model for TPO kinetics. Free TPO in plasma (TPO_f, V_p) binds to its c-Mpl receptor on platelet surface (R_p) to form drug-receptor complex (TPO_p) and is eliminated at the first-order rate (k_{el}). TPO_p dissociates back to free TPO and receptor or is internalized into platelet (k_{int}). TPO_f also has nonspecific tissue binding (A_{TPOn}, V_n).

A nonspecific TPO binding component (A_{TPOn}) is also present in the model to which TPO nonspecifically binds (k_{fn}) and dissociates (k_{nf}) at first-order rates. The nonspecific binding sites could be located in peripheral tissues.[17] In addition, we postulate the first-order TPO elimination (k_{el}) that can be attributed to renal and hepatic TPO clearances. However, this process is considered secondary to TPO receptor binding and internalization. The whole model is described by the following equations:

$$\frac{dTPO_f}{dt} = k_t - k_{on} \cdot TPO_f \cdot R_p + k_{off} \cdot TPO_p - k_{fn} \cdot TPO_f + k_{nf} \cdot A_{TPOn} / V_p - k_{el} TPO_f \quad (2)$$

$$\frac{dA_{TPOn}}{dt} = k_{fn} \cdot TPO_f \cdot V_p - k_{nf} \cdot A_{TPOn} \quad (3)$$

$$\frac{dTPO_p}{dt} = k_{on} \cdot TPO_f \cdot R_p - (k_{off} + k_{int}) \cdot TPO_p \quad (4)$$

$$\frac{dR_p}{dt} = k_r \cdot TPO_p / TPO_{p,0} - k_{on} \cdot TPO_f \cdot R_p + k_{off} \cdot TPO_p \quad (5)$$

where V_p denotes the plasma volume.

The endogenous TPO concentrations were set as the initial condition for TPO_f ($TPO_{f,0}$ = 1.4 pM).[23] The initial value of TPO bound to c-Mpl receptor ($TPO_{p,0}$), nonspecific binding sites ($A_{TPOn,0}$) and the zero-order rate con-

stants k_t and k_r can be calculated from the steady-state equations as follows:

$$TPO_{p,0} = \frac{k_{on} \cdot TPO_{f,0} \cdot R_{p,0}}{k_{off} + k_{\text{int}}} \quad (6)$$

$$A_{TPOn,0} = \frac{k_{fn} \cdot TPO_{f,0} \cdot V_p}{k_{nf}} \quad (7)$$

$$k_t = k_{int} \cdot TPO_{p,0} + k_{el} \cdot TPO_{f0} \quad (8)$$

$$k_r = k_t \quad (9)$$

where $R_{p,0}$ denotes the baseline c-Mpl plasma receptor concentration, which can be identified as the binding capacity of endogenous receptors. Equation 9 is the consequence of the simplified stimulatory mechanism described by Equation 1. Equations 6-9 hold only for the baseline (predose) conditions.

The model was applied to literature data. The data sets were obtained from the original article by Vadhan-Raj et al[23] via data digitization using the Sigma Scan program (Jandel Scientific Inc, San Rafeal, CA). A phase 1 and 2 cohort clinical study was performed by the investigators, and a single dose of recombinant human TPO was given intravenously to 12 patients with sarcoma (7 men and 5 women) prior to chemotherapy. Three patients were assigned to each of 4 dose levels (0.3, 0.6, 1.2, and 2.4 μg/kg of body weight). The free TPO concentrations in plasma were measured up to 5 days. Group mean data were used in the data analysis.

In order to develop mass balance equations, the concentrations of TPO were transferred from ng/mL to pM assuming the molecular weight of TPO was 70 kD. The average body weight of 70 kg was used in both noncompartmental and compartmental analysis.

Noncompartmental Analysis

A noncompartmental approach was used to identify the nonlinearity of TPO kinetics. Since TPO has a baseline level when no drug is administered, the baseline value (1.4 pM) must be subtracted from the measured concentration to carry out noncompartmental analysis. The noncompartmental analysis was performed using WinNonlin Professional release 2.1 (Pharsight Corp, Apex, NC). The C_{max}, T_{max}, steady-state volume of distribution (V_{ss}), apparent clearance, terminal slope (λ_z), terminal half-life, total area under the curve (AUC), area under the moment TPO_f versus time curve (AUMC), and mean residence time (MRT) were calculated. The C_{max} and T_{max} values were read directly from original data graphs. Terminal half-life was calculated as $0.693/\lambda_z$. The AUC and AUMC were calculated by the log-linear trapezoidal method with terminal phase extrapolation. The analysis was performed separately for different doses.

In order to analyze the linearity of TPO pharmacokinetics, dose normalization and the rule of superpositioning were used for evaluation. The dose-normalized concentration vs time and total AUC vs dose graphs were the major diagnostic tools.

Nonlinear Regression Analysis

The proposed pharmacokinetic model was simultaneously fitted to 4 dose data sets. The nonlinear regression was performed using ADAPT II release 4 program (Biomedical Simulations Resource, University of Southern California, Los Angeles, CA).[24] The maximum likelihood objective function was used to obtain system parameter estimates and the variance model shown below was applied:

$$Var(t) = \left[\sigma_{inter} + \sigma_{slope} \cdot Y(t)\right]^2 \quad (10)$$

where *Var(t)* is the variance of output at time t and *Y(t)* is the model output at time t. Two variance parameters σ_{slope} and σ_{inter} represent the linear relationship between SD of model output and Y(t).

The $TPO_{f,0}$ was fixed at 1.4 pM[23]. The initial estimate of baseline level of free receptor $R_{p,0}$ was calculated as 40 pM assuming the baseline level of platelets at 250×10^9 platelet/L and 100 receptor/platelet[20] according to the following equation:

$$R_{p,0} = PLT_0 \times (\#c - Mpl\ receptors\ per\ platelet)/6 \cdot 10^{23} \quad (11)$$

The initial estimates of k_{on}, k_{off}, and k_{int}, were obtained from the study by Li et al.[19] In the model, k_{on} and k_{off} were estimated independently. The V_p was fixed as plasma volume of 3 L. Then $TPO_{p,0}$, $A_{TPOn,0}$, k_t, and k_r were calculated from Equations 6, 7, 8, and 9 as secondary parameters. The fitted parameters were k_{on}, k_{off}, k_{int}, k_{nf}, k_{fn}, and $R_{p,0}$.

RESULTS

Noncompartmental Analysis

Two graphical methods are used to evaluate the nonlinearity of TPO disposition. First, dose normalized TPO_f concentrations are plotted vs time for each dose (Figure 2). Judging by the rule of superposition, nonlinear phar-

Table 1. Noncompartmental Pharmacokinetic Parameters for Thrombopoietin Using Patient Group Mean Data*

Parameter	Dose μg/kg			
	0.3	0.6	1.2	2.4
T_{max} (hour)	0.08	0.03	0.17	1
C_{max} (μg/kg)	5.0	11.6	17.2	40.2
λ_z (1/h)	0.020	0.028	0.031	0.037
$t_{1/2-\lambda z}$ (hour)	35.6	24.7	22.1	18.5
CL (L/h)	0.73	0.86	0.48	0.30
V_{ss} (L)	22.4	14.5	12.3	6.0
AUC (μg·h/L)	28.9	48.9	176.5	558.8
AUMC (μg·h^2/L)	889.9	826.2	4548.3	11192.6
MRT (hour)	30.8	16.9	25.8	20.0

*AUC indicates area under the curve; AUMC indicates area under the moment TPO_f versus time curve; and MRT, mean residence time.

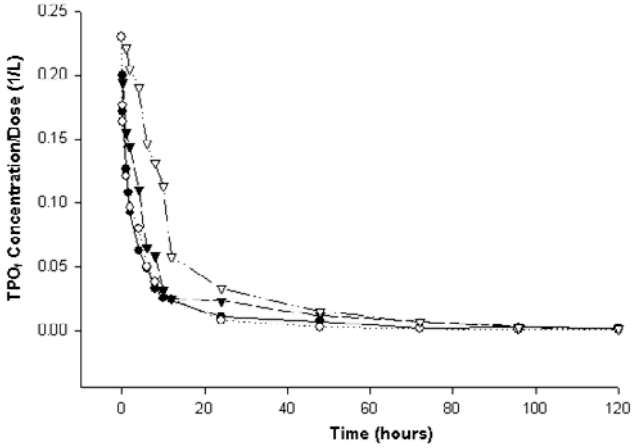

Figure 2. Free TPO plasma concentration/dose vs time plot for each dose. Symbols represent data from Vadhan-Raj et al.[23] Dose levels: 0.3 (●), 0.6(○), 1.2(▼), and 2.4 (▽) μg/kg.

macokinetics could be concluded by visual inspection because the plots from the 4 dose levels do not converge into one. Second, total AUC for each of the 4 doses are plotted vs dose. Nonlinear pharmacokinetics is also indicated by the curvature of the plotted line (Figure 3). Parameters obtained from noncompartmental analysis are summarized in Table 1. As dose increased, λ_z increased accordingly and total AUC increased in a more than dose-proportional manner, while clearance (CL) and V_{ss} go to the opposite direction.

Nonlinear Regression Analysis

The model Equations 2 through 5 were simultaneously fitted to plasma-free TPO data and the fittings are shown in Figure 4. The pharmacokinetic profile of TPO exhibits multiexponential behavior. The estimated parameters are shown in Table 2. The model allowed the estimation of the binding parameters k_{on} and k_{off} as well as internalization parameter k_{int}, which are of major interest. They were estimated with a good precision with the coefficient of variation (CV) not exceeding 37%. The estimated k_{on} value is similar with results from in-vitro binding analysis.[21] In addition, the endogenous platelet-binding capacity $R_{p,0}$ was estimated as 164.0 pM, which yielded the calculated number of receptors per platelet 398, if 250×10^9 platelets/L is assumed. The internalization half-life calculated from k_{int} was 6.9 hours. The baseline values of $A_{TPOn,0}$ and $TPO_{p,0}$ were estimated as 11.11 pmol (CV = 13.6%) and 4.87 pM (CV = 15.8%). The zero-order production rate for endogenous free TPO (k_t) and c-Mpl receptor k_r were both estimated as 0.49 pM/h (CV = 10.7%). The elimination rate k_{el} was fixed at 0.

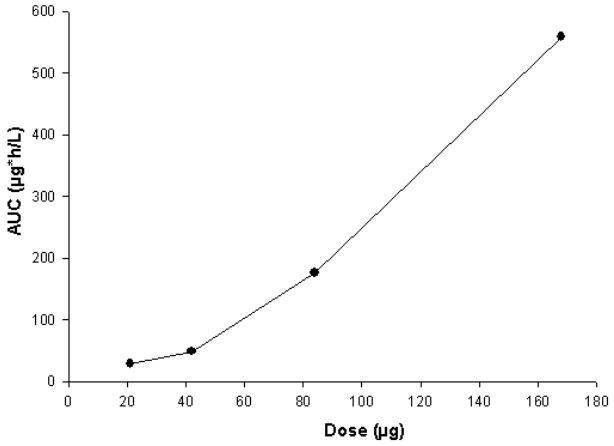

Figure 3. Superposition of AUC vs dose plot. Solid symbols represent AUC values for 4 doses. Dose levels: 0.3, 0.6, 1.2, and 2.4 μg/kg.

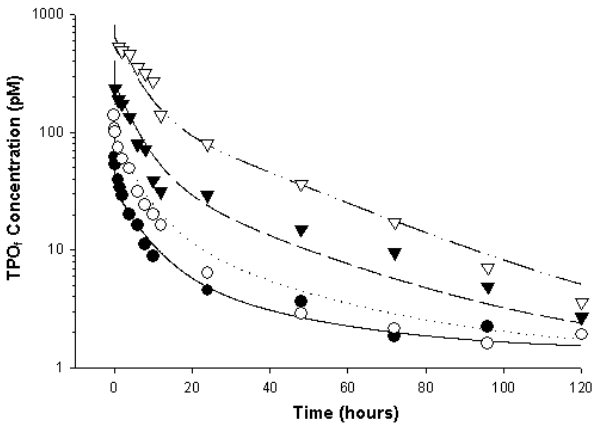

Figure 4. Time-course of TPO plasma concentration with model fitting. Symbols represent clinical data from Vadhan-Raj et al.[23] Dose levels: 0.3 (●), 0.6(○), 1.2(▼), and 2.4 (▽) μg/kg. Solid lines represent profiles predicted by the presented model (Equations 2-5).

Table 2. Final Pharmacokinetic Parameter Estimates for Thrombopoietin Using Patient Group Mean Data*

Parameter (Unit)	Estimate	CV (%)
k_{on} ($pM^{-1}h^{-1}$)	0.055	36.2
k_{off} (h^{-1})	2.50	35.1
k_{int} (h^{-1})	0.10	11.1
k_{nf} (h^{-1})	0.048	17.5
k_{fn} (h^{-1})	0.13	14.0
$R_{p,0}$ (pM)	164.0	15.3
k_{el} (h^{-1})	0†	

*CV indicates coefficient of variation.
†Parameter was fixed.

The simultaneous fittings resulted in partial overestimation of the 0.6 and underestimation of the 1.2 μg/kg dose group, whereas other dose groups were fitted reasonably well. Overall, a good agreement between the observed and predicted data was observed.

DISCUSSION

Model Building

TPO metabolism and elimination have been extensively studied in vitro, and it is widely accepted that binding to c-Mpl receptors on platelets is the major elimination pathway of TPO. It has also been demonstrated that there is a reciprocal relationship between plasma TPO level and platelet mass. With limited numbers of c-Mpl receptors residing on platelet and megakaryocyte surfaces, if a high dose of TPO is administered, the concentration-time profile of TPO would exhibit capacity-limited and nonlinear characteristics. Such behavior could be categorized as target-mediated drug disposition, a term introduced by Levy[25] to describe a process in which drug binds to its specific receptor and subsequent metabolism and degradation become its primary route of elimination. The proposed pharmacokinetic model is built on such theory to characterize TPO disposition. We adopted the pharmacokinetic model of target-mediated disposition introduced by Mager at al.[19] The only change we made was the incorporation into our model of the free receptor compartment R_p, whereas originally the free receptors were expressed as R_{max} – DR, where R_{max} denoted the total concentration of receptors and DR was the concentration of the drug-target complex. The free receptors R_p and bound receptors TPO_p in our model have independent kinetics, and the total number of receptors is the sum of these 2: $R_{max} = R_p + TPO_p$.

The binding of TPO to its receptor c-Mpl is a crucial component of the whole model. The TPO binding properties have been studied intensively with a reported binding capacity ranging from 25 to 225 receptors per platelet and binding constant K_d values ranging from 100 to 846 pM indicating a very high binding affinity.[20] In addition to platelets, c-Mpl is also expressed on megakaryocytes and to a much less extent, on progenitor cells such as CD34+ cells.[7] The binding of TPO to receptors on these cells is not included in the model for several reasons. For progenitor cells, the expression of c-Mpl on these cells is very low and their contribution to TPO kinetics is insignificant. For megakaryocytes, the expression of c-Mpl is also minor compared with platelets, and it will not affect the general pharmacokinetic profile of TPO very much. Another reason is that megakaryocyte is a giant cell with heterogeneous ploidy in nature.[26] The receptor number on one megakaryocyte is distinct in relation to others. However, since the binding of TPO to megakaryocytes is the initiating event that stimulates the production of platelets, this compartment should be incorporated into the model if the pharmacodynamic effect of TPO is also of interest.

Upon binding, the drug-receptor complexes are susceptible to internalization into platelets and degradation by lysosomes. Such a phenomenon is called receptor-mediated endocytosis, which constitutes the principal pathway for the irreversible removal of TPO from the body. After degradation, the receptors would not recycle back to the platelet surface.

As a glycoprotein with 353 amino acids (70 kD), TPO is predominantly distributed in the circulation system. The high molecular weight also precludes its transport to peripheral tissues extensively. Thus the volume for the free TPO compartment is fixed to be the plasma volume (3 L for a normal person of weight 70 kg). TPO has a very long half-life in the systemic circulation. The 177

amino acid carboxyl terminal domain retaining several N-linked glycosylation sites are believed to stabilize TPO and prevent it from degradation by proteases and other enzymes.[12] It might be inferred that hepatic metabolism and renal clearance have minor contributions to total TPO elimination. Large molecular weight limits the renal elimination of TPO because a macromolecule with a molecular weight larger than 69 kD is unlikely to traverse the glomerulus.[27] The C-terminal glycosylation also prevents the molecule from readily transporting into liver. Therefore, the elimination directly from the free TPO compartment is ignored ($k_{el} = 0$).

A nonspecific binding compartment is also included in the model. Fielder et al[17] have demonstrated that TPO is distributed into several tissues, especially highly perfused tissues, in mice after exogenous TPO administration. Consequently, we found it is necessary to incorporate this compartment into the model.

The other feature of this model is the presence of a stimulation process on c-Mpl receptor production. Based on its mechanism of action, the input of exogenous TPO would increase the production of platelets. Therefore, more receptors would be available for binding after drug administration. The extent of platelet production increase is dependent on how much TPO has bound to c-Mpl receptors on megakaryocytes. It has been reported that c-Mpl receptors on platelets and megakaryocytes have the same binding characteristics.[17] To avoid addition of the megakaryocyte compartment into the model, a simplistic assumption was made that the TPO bound to receptors on megakaryocytes is reflected as part of TPO bound to platelets. In terms of the pattern for a stimulation process, a direct proportion of $TPO_p/TPO_{p,0}$ was used. Such a stimulation term is highly sensitive to the concentration change of TPO_p and can produce a sharp increase of production rate. It could be otherwise modified to a slow stimulation term when a more delayed effect is expected. The nonlinear Hill equation would be the best candidate for such a process, since for higher TPO concentrations, a saturation of the stimulus can be expected. However, to minimize the number of model parameters, the linear stimulation was assumed. By controlling the nature of the stimulation process, we can get various pharmacokinetic profiles of TPO bound to platelets and/or megakaryocytes. Such information could be used as the driving force for the indirect response model[28] or life-span model[29] established to characterize the pharmacodynamic effect of TPO.

A clinical data set from Vadhan-Raj et al[23] was used to evaluate the proposed model. The patients recruited in the clinical study had normal hematopoietic function before treatment. Their blood cell counts, with the exception of platelets, were before and remained within the normal limits after TPO treatment. Only platelet levels were elevated by day 21 but before the initiation of chemotherapy. Hematological alterations can be observed in as many as 40% of patients with soft tissue sarcomas prior to chemotherapy[30]. Thrombocytosis can occur in 15% of patients. The frequency of hematological abnormalities increases in patients with advanced tumors. The study encompassed 4 doses varying from 0.3 to 2.4 μg/kg. These levels are sufficiently large for assessing nonlinear pharmacokinetics drug modeling.

Noncompartmental Analysis

After drug input, the plasma-free TPO concentrations reach the maximum value and increase in a dose-related manner. Then the concentrations drop very quickly for the 2 lower doses; this is less apparent for the 2 higher doses indicating a certain elimination or distribution threshold has been approached for high doses. The terminal slope increases as the dose increases, rendering the terminal half-life ranging from 18 to 35 hours. The enzyme-linked immunosorbent assay (ELISA) quantitative method for TPO measurement has a general detection limit at 0.15 ng/mL, which is close to the TPO endogenous level.[31] The concentrations for the terminal phase for the 2 lower doses may have already reached baseline, thus the terminal half-life may be overestimated. The apparent drug clearance is low for high doses, indicating the elimination processes may have been saturated at high dose levels. The apparent steady-state volume of distribution decreases as well when the dose is increased confirming nonlinearity. Furthermore, it has also been demonstrated that saturable plasma protein binding is insignificant regarding specific drug target binding or tissue binding.[32] However, caution should be kept in mind when interpreting noncompartmental results because the CL and V_{ss} calculations may be erroneous when a nonlinear elimination process is involved.[33] The dose dependence of CL and V_{ss} is a consequence of the nonlinear receptor-mediated kinetics. This phenomenon has been analyzed elsewere.[19]

Two graphical techniques were used to identify the nonlinearity of TPO pharmacokinetics. When the concentrations divided by dose were plotted vs time for each dose, the lack of superposition for the 4 doses indicates the existence of dose-dependent pharmacokinetics. When AUC values were plotted vs dose, it was observed that AUC values were not proportional to dose. This finding also indicates nonlinear pharmacokinetics.[34] Moreover, an increase in AUC, which is greater than dose proportional is very much likely attributable to saturation in elimination.[35]

Nonlinear Regression Analysis

The proposed model was employed to describe the clinical data set. The predicted profile captured the data points for all dose levels relatively well. The reported parameters had relatively low coefficients of variation. The estimated k_{on} value was similar to the initial estimate taken from literature.[21] The calculated K_d value (45.5 pM) from estimated k_{on} and k_{off} value was, however, lower than the reported value (100-846 pM). There are several possible reasons that could account for this discrepancy. The K_d value is often obtained in vitro. The binding study of Li et al[21] was performed in a 25°C system, which is different from the normal physiological condition. Also in their study, they found that temperature greatly affected the clearance of TPO. TPO clearance at 4°C was only 2% of that at 37°C.

The value of $R_{p,0}$ predicted by our model, on the other hand, is greater than the initial value. The $R_{p,0}$ represents the total platelet binding capacity at physiological state, and the deviation of high final estimate as 164.0 pM from the initial value was partially due to the exclusion of megakaryocytes, which carry several receptors. The platelet baseline count and receptor density have high variations in the literature, which make the initial estimate less accurate. However, the estimated binding capacity is comparable with the reported binding capacity as 64 pmol per liter of blood by Fielder et al.[20] In addition, the zero-order production rate for R_p (k_r) was calculated as 0.49 pM/h. If the platelet life-span is considered as 10 days, the binding capacity of TPO to c-Mpl based on the natural turnover of platelets will be calculated as 120 pM, which is close to the model estimate.

An alternative model without a stimulation process has been developed and compared with the proposed model. The sum of squared residuals, Akaike Information criteria and Schwartz criteria implicated that such a stimulation process is necessary for the model. Furthermore, the estimated k_{nf} value from this model is approaching zero, resulting in an unreasonably high steady-state concentration for nonspecifically bound TPO. Another model with the linear elimination process (k_{el}) from the central compartment was also established to check if renal or hepatic clearance is necessary. The fitting resulted in a very small k_{el} value close to zero, and no improvement in fitting occurred, indicating that such a component may not be necessary. In addition, a model without the nonspecific binding compartment was examined. From the model output, the estimated $R_{p,0}$ value was 493.5 pM, which would result in a receptor density of 1200 c-Mpl per platelet. Consequently, the proposed model provided both the best fittings and physiological meaningful parameters.

CONCLUSION

A target-mediated endocytosis disposition model has been established to account for the nonlinear characteristics of TPO pharmacokinetics. The model well captured the concentration-time profiles of the clinical data set after single-dose administration of TPO in patients with sarcoma prior to chemotherapy. The determined binding and internalization parameters are comparable with reported results from in vitro studies. Future studies will be focused on the application of the present model to the modeling of pharmacokinetic (free TPO plasma concentration) and pharmacodynamic data (platelet count) simultaneously.

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